
The effect of BCG vaccine at birth on the development of atopy or allergic disease in young children

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Background: Exposure to infectious diseases may reduce the development of asthma or allergy. In particular, the role of the BCG vaccine in modulating asthma or allergy has been a source of speculation.

Objective: To study newborns from 3 international sites to evaluate the prospective effect of BCG vaccine on allergic diseases or atopic development.

Methods: Infants were enrolled from newborn and well-infant clinics in Thailand, Argentina, and Turkey. The standard BCG vaccine for each country was given at birth. Parents who consented to have their infant included in the protocol completed an allergy family questionnaire. Infants underwent a standard purified protein derivative (PPD) test at 9 to 12 months of age, and the reaction size was measured. At the age of 2 years, the children returned to be studied. Allergy skin tests to common allergens appropriate to location and age were performed, and the parents completed the International Study of Allergy and Asthma in Childhood questionnaire. The PPD reaction size was compared with the presence of atopy and allergy questionnaire responses.

Results: A total of 1,704 infants were studied. Statistical significance was found between a negative PPD response vs any positive PPD response and the risk of having an allergic history at the age of 2 years in Turkey (relative risk, 2.11; 95% confidence interval, 1.25–3.55; $P = .005$) and Thailand (relative risk, 2.16; 95% confidence interval, 1.18–3.94; $P = .02$) but not Argentina (relative risk, 1.09; 95% confidence interval, 0.70–1.68; $P = .70$).

Conclusions: This study further supports the role of infectious agents in modulating asthma and allergy development.

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INTRODUCTION

Asthma affects at least 15 million people in the United States and is currently the most common chronic childhood disease.¹ Although a number of hypotheses have been proposed to cause this epidemic of asthma, the current prevailing hypothesis is the effect the absence of infectious stimuli might have on development of atopic disease.^{2,3} The “hygiene hypothesis” is based on the supposition that the decline of infectious disease in the developed world is associated with an immune imbalance, with a resultant increase in the development of allergy.^{3–5} A T_H2 skewed response characterizes newborns, and the failure to promote an interferon- γ response to bacterial antigens may prolong the period during which T_H2 responses are predominant.⁶ These findings are also supported by the work of Shirawaka et al,⁷ in which children in Japan who were vaccinated with BCG and who had a positive response to purified protein derivative (PPD) were less prone to atopy and allergic disease.

The BCG vaccine has proved to be an effective immunomodulator on the development of allergic pulmonary responses in a murine model of asthma. Both as a preliminary treatment^{8,9} and as a post-allergen exposure treatment,¹⁰ the BCG vaccine can attenuate specific components of the murine allergic pulmonary response. In mice, BCG vaccination results in a preferential proliferation of T_H1 cells, with the production of type 1 cytokines and interleukin 12.^{11–15} These studies point to a potential mechanism for the antiasthmatic effects of microbial infection in childhood.

These observations have generated interest in BCG as an immune modulator in humans. The BCG vaccination is used in many countries as a primary tuberculosis prophylaxis; however, it is not used in many developed nations, including the United States. As asthma prevalence has increased during the last decade in developed countries, the use of the BCG vaccine as a primary modulator of asthma and allergy has gained support.

This study aimed to prospectively determine whether there is an association between the skin test response and allergic disease in populations that routinely receive the BCG vaccine at birth. Our collaborators were located at 3 international centers.

METHODS

Infants were enrolled at birth without regard to allergy and asthma history. Infants were enrolled at birth or within 2

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months after birth at clinics associated with the university hospitals in Argentina, Thailand, and Turkey.

Marmara University Hospital is the teaching hospital for the Marmara University School of Medicine and is located in Istanbul, Turkey. The infants enrolled from Marmara University were healthy newborns enrolled consecutively from births at the University Hospital. Infants born prematurely, those with major illness, or those admitted to the newborn intensive care unit were excluded. In Turkey, when the infant had a primary care physician, the physician was contacted to be aware of the protocol and to enlist his or her assistance in performing the required PPD challenge. For reference, an International Study of Asthma and Allergies in Childhood (ISAAC) analysis of wheezing and rhinitis in 2,276 children 6 to 15 years of age in Istanbul revealed a current prevalence for wheezing of 7.2% and a cumulative prevalence of allergic rhinitis of 17.6%.¹⁶

Newborn infants enrolled in the Thailand study were from the newborn nursery at Siriraj Hospital, the largest University Hospital in Bangkok, with more than 300 pediatric in-hospital beds, or were recruited from the university well-infant clinic at their first postbirth visit. Infants with perinatal complications or with significant illnesses before enrollment were excluded. As previously published,¹⁷ the parent-completed ISAAC questionnaire data for asthma showed that 11% of 3,629 children aged 6 to 7 years in Bangkok had current wheeze and 9.3% "ever had asthma." The investigators used the ISAAC questionnaire to evaluate rhinitis and eczema in Bangkok. Their study revealed a 12-month prevalence of rhinitis in children ages 6 to 7 years and 13 to 14 years of 38.7% and a 14.0% prevalence of eczema.¹⁸

In Cordoba, Argentina, infants were enrolled from the municipal preventive care center at their initial postbirth visit. The center is located in downtown Cordoba, a city with a population of 1 million, in central Argentina. Infants were excluded if they had significant perinatal illnesses, prematurity, or an intensive care admission. For reference, the ISAAC questionnaire on asthma and wheezing in Argentina showed that in more than 6,000 children 6 to 7 years of age current wheeze was present 16% of the time but "ever had asthma" was answered less frequently at 5.3%.¹⁷ The self-reported current prevalence of allergic rhinoconjunctivitis in Argentinian children aged 6 to 7 years was approximately 17.5%¹⁹ and the prevalence for current atopic eczema was approximately 8%.²⁰ The investigators had previously reported on the prevalence of atopic dermatitis in 6-year-old children in Cordoba, Argentina, using the ISAAC questionnaire.²¹ The questionnaire identified that 9.6% of 498 children in first grade reported an itchy rash in the past year.

Parents of enrolled infants received and signed a consent form in Thailand; in Turkey and Argentina, only verbal consent was required. Because the collaborating countries require each newborn to be vaccinated within the first 2 months of life, all infants were administered a routine BCG vaccine using a standardized immunization protocol and according to national and local health practices in each country.

In Thailand, the BCG vaccine was obtained from the Thai Red Cross Society (Queen Soavabha Memorial Institute, Bangkok, Thailand). Freeze-dried BCG vaccine of Pasteur Merieux (Lyon, France) was used in Turkey, and in Argentina the BCG source was BCG Pasteur-Merieux (strain 1173 P2). A family pedigree and family allergy history were obtained at enrollment. Routine medical care for the enrolled infants was administered by their family physicians or at the university-based clinic. All data were entered in a database.

Enrolled infants between the ages of 9 and 12 months were tested with 5 U of PPD of *Mycobacterium bovis* (Mantoux test). The skin test solution was performed intradermally on the volar surface of the forearm, producing a 5 × 5-mm wheal immediately. The PPD skin test response was measured 48 to 72 hours using the "ball point pen" method. The size of the PPD measured response was entered into a database. The PPD skin test results were stratified using millimeter induration increments. The BCG scar size results were also stratified using millimeter scar increments.

Parents of the enrolled children were administered the ISAAC questionnaire²² when their child was 2 years old, with the questionnaire translated into the language of each country. The ISAAC questionnaire is a well standardized tool for determining allergic and asthmatic histories. The ISAAC questionnaire contains core questions for wheezing and asthma, rhinitis, and eczema.²² The questions for our study duplicated the ISAAC parental questions for 6- to 7-year-old children. Positive responses to questions for wheezing (asthma-like) were allowed, as was the question directly relating to asthma itself; similarly for rhinitis, positive responses for nasal symptoms (hay fever-like) apart from colds were allowed, as was the question for hay fever itself; and for eczema, the questions for itchy rash were allowed, along with a positive response to eczema. A 2-year-old was considered allergic if responses to any of these questions were positive. The question "Has your child had wheezing or whistling in the chest in the last 12 months?" has been validated for asthma.²³ The questions characteristic for hay fever have been validated.¹⁹ Questions for symptoms compatible with atopic dermatitis have been validated.²⁰

Skin tests for food and indoor and outdoor allergens and control tests were performed. Allergy skin test vials were supplied by Hollister-Stier (Spokane, WA) and included house dust mites (2 species), cockroach mix, *Alternaria*, cat dander, dog dander, egg, milk, shrimp (Thailand), Bermuda grass (Thailand), Johnson grass (Thailand), *Cladosporium*, and *Aspergillus* species (Turkey). Skin prick tests were performed with positive and negative controls (histamine and saline, respectively) using a lancet on the infants' volar surface of the forearm or the upper back. Atopy was defined as a positive wheal response 2 mm in diameter or greater than the saline control. Information on skin tests and questionnaire data were entered into a database.

Statistical analysis of our enrolled cohorts included a crude odds ratio (OR)²⁴ for the development of both atopy and atopic symptoms at the age of 2 years in those who responded

positively vs negatively on PPD skin tests. Statistical significance was determined by χ^2 analysis and set at $P < .05$. The ORs are shown with 95% confidence intervals (CIs).²⁴ The BCG scar size was independently analyzed as a predictor of atopy. Analysis was performed on results from each country individually and not pooled.

RESULTS

A total of 1,704 newborns were enrolled. Table 1 presents the enrollment numbers, with parental allergy and asthma and maternal smoking information. Table 2 outlines the number of children who underwent a PPD test at the age of 9 to 12 months and those who were then screened at the age of 2 years with the ISAAC questionnaire and who underwent an allergy skin test battery. The PPD skin test induration was stratified. A summary of the PPD size for the infants at the 3 sites is shown in Table 3.

For each international site, multiple stratifications were analyzed for the effect of PPD response at 9 to 12 months on the development of allergic histories (see the "Methods" section) or positive allergy skin test results. Statistical significance was found between a negative PPD response vs any positive PPD response and the risk of having an allergic history at the age of 2 years in Turkey and Thailand but not Argentina (Table 4).

In a separate analyses (data not presented), there was no association between PPD size for specific diagnoses of eczema, hay fever, or asthma (based on a positive answer to "Has your child ever had asthma, or hay fever, or eczema?") due to the small number of children at the age of 2 years with a physician diagnosis. A similar analysis showed that there was no stratification of BCG scar size that was associated with the presence or absence of having an allergic history, a specific diagnosis, or positive allergy skin test results.

Recruitment of newborns was accomplished at the medical centers or university-associated well-infant (immunization) clinics. No attempt was made to recruit infants with an enriched allergic or asthmatic history. For that reason, the returning children at the age of 2 years were limited by parental interest and the investigator's continued accessibility to the population following enrollment. We analyzed the

Table 1. Parental Histories for Each Center

	No. of infants		
	Thailand	Turkey	Argentina
Enrolled	550	604	550
Mother with asthma	15	11	53*
Mother with hay fever	44	30	89*
Father with asthma	18	16	53*
Father with hay fever	40	19	83*
Both with asthma	0	1	3
Mother smoked during pregnancy	17†	68	62

* Significantly higher than each of the other 2 countries.

† Significantly lower than each of the other 2 countries.

Table 2. Enrollment of Infants and Follow-up

	No. of infants		
	Thailand	Turkey	Argentina
Enrolled	550	604	550
PPD test	321	265	183
Year 2 visit	233	241	181

Abbreviation: PPD, purified protein derivative.

Table 3. Stratification of PPD Size for Each Center

Induration size, mm	No. of infants		
	Thailand	Turkey	Argentina
0	98	51	43
0.1–4.9	30	29	54
5.0–6.9	31	101	52
7.0–9.9	52	57	13
10.0–14.9	14	3	19
>15	8	0	0
Total	233	241	181

Abbreviation: PPD, purified protein derivative.

Table 4. Crude Relative Risks for Atopy and Allergic History Based on PPD Positivity

Country	Relative risk (95% CI)			
	Atopic (if PPD negative)	P value	Allergic history (if PPD negative)	P value
Thailand	1.13 (0.59–2.15)	.70	2.16 (1.18–3.94)	.02
Turkey	1.65 (0.89–3.04)	.15	2.11 (1.25–3.55)	.005
Argentina	1.10 (0.59–2.05)	.75	1.09 (0.70–1.68)	.70

Abbreviations: CI, confidence interval; PPD, purified protein derivative.

family allergic status in those families who returned for the year 2 visit compared with those families who could not be retrieved for follow-up. There was a significant increased rate of return for follow-up in children whose mother was asthmatic in Argentina (OR, 1.87; 95% CI, 1.06–3.31; $P = .02$) and if there was a current indoor smoker in Thailand (OR, 1.47; 95% CI, 1.10–1.95; $P < .001$). The higher asthma prevalence of parents from Argentina (Table 1) suggests an enrichment effect, which may have negated any potential of BCG to attenuate the development of allergic disease in their offspring.

DISCUSSION

In humans, retrospective studies of the BCG vaccine as an immune modulator have proved controversial. Studies have suggested a protective effect,^{7,25,26} a weak protective effect,²⁷ no effect,^{28–34} or a temporary effect.³⁵

In Japan, children with positive tuberculin (PPD) skin test results, induced by BCG vaccination at birth, have reduced asthma incidence and allergen sensitization.⁷ A negative relationship between delayed hypersensitivity to tuberculin re-

sponses and the presence of asthma was demonstrated. Children with a positive delayed hypersensitivity to tuberculin skin test response had serum cytokine concentrations suggestive of a predominant T_H1 response, in contrast to the T_H2 profiles seen in children with a negative tuberculin skin test result.⁷ In another study, Aaby and colleagues²⁵ reported that BCG vaccination during the first week of life is associated with a decreased risk of atopy in children in Guinea-Bissau. These investigators found that when the BCG vaccine was not given until after 6 months of life, there was no significant decreased risk of atopy in children in Guinea-Bissau. In a separate study in Germany, BCG vaccinated children showed lower rates of atopic sensitization and clinical manifestations before the second year of life, and PPD response was not decreased in atopic children.³⁵

In contrast to the studies by Shirakawa et al⁷ and Aaby et al,²⁵ Alm et al²⁸ investigated whether BCG vaccination against tuberculosis influenced the development of atopy in Sweden. They found that early BCG vaccination in children with atopic heredity did not affect the development of atopic diseases. Similar findings were reported by workers in Norway.²⁹

In the retrospective study by Alm et al,²⁸ it is not clear whether the children with atopic heredity were recruited from high-risk allergy families or low-risk allergy families, and there was no skin testing performed to rule out the existence of any atopy. The differences between the studies reported by Shirakawa et al⁷ and others could be due to the difference in timing of BCG vaccination. Whereas the subjects in the study by Shirakawa et al were immunized at birth or younger than 2 months, those subjects studied by Alm et al²⁸ and Omenaas et al²⁹ were immunized as late as 6 months of age and those in the study by Jentoft et al³² were immunized at 14 years of age. Because the incidence of tuberculosis is approximately 10 times greater in Japan compared with the Scandinavian countries, exposure of children to environmental mycobacteria was not similar. Other environmental mycobacteria could also contribute to the differences.

The timing of BCG vaccination may be of central importance, because studies have indicated that a "window of opportunity" may exist for influencing T_H1/T_H2 balance³⁶ or even intrauterine balance.³⁷ Newborns have an immune response that is tilted toward a T_H2 response.⁶ However, infants vaccinated with BCG at birth or 2 months develop a T_H1 response against BCG antigens.³⁸

Other confounding factors that could also induce different responses in T cells and macrophages are the different strains of BCG vaccine.³⁹ Furthermore, the presence of other environmental mycobacteria clearly can induce a difference in the protective ability of BCG as mentioned previously.

The central issue addressed by our study was asked by Krishna and Salvi⁴⁰ in a recent review: "Could administration of bacille Calmette-Guerin vaccination at birth protect from the development of asthma and allergic diseases in the western world? Has this question been adequately investigated?" In a prospective study of the question, we have shown a protective effect in young children of the BCG vaccine

against the development of allergic symptoms, but not atopy, in 2 international centers.

Controversy still remains regarding the study design of the effectiveness of the BCG vaccine in preventing the development of allergic disease. Our protocol, for example, does not use a placebo BCG group. Since we were unable to modify the public health policy in our collaborative countries, we chose as a reasonable surrogate a negative PPD result (despite BCG). It is unlikely that a large placebo BCG group will ever be available, largely due to public health limitations. In addition, the absence of a BCG scar would alert the parents of the actuality of the placebo, although other studies have used placebo BCG vaccine in adults without apparent bias.⁴¹

Choosing an instrument to define allergy and/or atopy in young children in an epidemiologic study remains controversial. We selected the ISAAC questionnaire as a well known and validated instrument to document the presence of allergic symptoms or allergic diseases in our study population. The ISAAC questionnaire is commonly used in 6- to 7- and 13- to 14-year-old children in its worldwide application. It is yet to be tested in younger children. However, the questions are still applicable and were used in all 3 centers. Since a specific allergic disease (eg, asthma) diagnosis is less often made in young children, we allowed allergic symptoms to be used in addition, an association that has been validated.^{19,20,23}

The value of using allergy skin tests in epidemiologic studies of young children can be questioned. In a large prospective study of the development of seasonal allergic rhinitis at 2 years of age, approximately 7.5% of enrolled children had rhinitis symptoms, but only 3% had a positive CAP-RAST-ELISA response (Pharmacia, Uppsala, Sweden) to birch or grass, and only 2.6% had both symptoms and specific sensitivity.⁴² This would argue a reduced benefit of allergy skin test response as a critical factor in determining the protective benefit of the BCG vaccine in young children.

In summary, our report adds to the information gained on the human use of BCG vaccine as a modulator of allergic disease. A recent report showed a therapeutic effect of the BCG vaccine in Korean adults with moderate-to-severe asthma,⁴¹ and a study demonstrated an effect on cytokine messenger RNA expression in children with asthma in Turkey.⁴³ These reports show continued interest in using the BCG vaccine in allergic disease attenuation or treatment.

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